Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 84-248 are pending in the application, with 84, 101, 117, 135, 153, 156, 159, 175, 191, 209, 212 and 215 being the independent claims. Claims 22-83 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Applicants reserve the right to pursue the subject matter of these claims in related applications.

The specification has been amended to conform to the formal drawings submitted herewith. New claims 84-248 are sought to be added. The new claims are presented to more particularly point out what Applicants regard as the invention, and/or to make explicit that which was implicit in the canceled claims. Support for the new claims may be found throughout the specification. The amendments do not introduce new matter, wne their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Restriction/Election

Applicants thank the Examiner for reconsidering the restriction requirement of July 6, 2001 (PTO File Wrapper Paper No. 12), and acknowledging it as a species election. Applicants hereby affirm the election, with traverse, made by telephone on November 5, 2001 to prosecute the invention of Group I as delineated by the Examiner in Paper No. 9,

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i.e., claims to an antibody which binds to the polypeptide of SEQ ID NO:2 (*i.e.*, amino acids 1 to 468 of SEQ ID NO:2), with a species election of an antibody to the polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2, or which binds to the extracellular domain of the polypeptide encoded by the cDNA clone in ATCC Deposit No. 97853. Applicants assert the right to claim additional species in the event that a generic claim thereto is found to be allowable in accordance with 37 C.F.R. § 1.141(a).

It is believed that new claims 84-157, 159-210, 212, 213, 215-232, and 248 presented herein correspond to this election, since antibodies or antibody fragments which specifically bind the DR4 extracellular domain are sub-generic to, and therefore overlapping in scope with, antibodies or antibody fragments which specifically bind the full-length DR4 polypeptide or the polypeptide encoded by the cDNA of ATCC Deposit No. 97853. To the extent that the Examiner requests that Applicants list the claims particularly directed to the provisionally elected species, Applicants point out that new claims 84, 101, 117, 135, 159, 175, 191 and 215 and dependent claims 85-100, 102-116, 118-134, 136-152, 160-174, 176-190, 192-208, 216-232, and 248 are particularly directed to the elected species. This election is made without prejudice to or disclaimer of the other claims or inventions disclosed.

Applicants respectfully traverse and request the withdrawal of the requirement for election of species. As a threshold matter, Applicants point out that MPEP § 803 lists the criteria for a proper restriction requirement:

> Under the statute an application may properly be required to be restricted to one of two or more claimed inventions only if they are able to support separate patents and they are either independent (MPEP § 806.04 – § 806.04(i)) or distinct (MPEP § 806.05 – § 806.05(i)).

> If the search and examination of an entire application can be made without serious burden, the examiner must examine it on the merits, even though it includes claims to independent or distinct inventions.

Thus, even assuming, *arguendo*, that the groups listed by the Examiner represent patentably distinct species, restriction remains improper unless it can be shown that the search and examination of both groups would entail a "serious burden." *See* M.P.E.P. § 803. In the present situation, no such showing has been made.

Applicants submit that a search of antibodies or fragments thereof which specifically bind the polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2, or which binds to the extracellular domain of the polypeptide encoded by the cDNA clone in ATCC Deposit No. 97853 would also provide useful information regarding antibodies or fragments thereof which specifically bind smaller fragments of the extracellular domain of the DR4 polypeptide, as well as other fragments of the full-length DR4 polypeptide. Indeed, since the claimed antibodies or fragments thereof bind to portions of the same sequence (SEQ ID NO:2), a search of each of them would largely, if not entirely, overlap. Thus, the search and examination of all of the claims of group I would not entail a serious burden.

Accordingly, reconsideration and withdrawal of the Requirement for Election of species, and consideration and allowance of all pending claims, are respectfully requested.

Objection to the Drawings

The Examiner has noted that the informal Figures 2A and 2B are duplicates of each other. Applicants submit herewith formal drawings, thereby rendering the objection moot. The specification has been amended throughout to conform to the numbering of the formal

drawings.

Objection to the Claims

The Examiner has noted that Claims 24 and 46 depend on non-elected claims. Claims 24 and 46 have been canceled, rendering this objection moot.

Rejections under 35 U.S.C. § 112, First Paragraph

The Examiner has rejected claims 39, 40, 61, 62, 70, 71, 79 and 80 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the relevant art to which it pertains, or with which it is most nearly connected, to make and use the invention. (Paper No. 15, page 4.) Specifically, the Examiner has stated that

> [b]ecause of the relatively low skill level in the art, lack of teachings in the specification and prior art about how the structure of the receptor is related to activation or inhibition of its receptor, lack of examples in the specification or prior art of anti-DDR antibodies, and more particularly, agonistic or antagonistic antibodies, the unpredictability of making an anti-ligand antibody which is an agonist or antagonist of the DR4 receptor, it would require undue experimentation to make the invention as claimed.

Id. Claims 39, 40, 61, 62, 70, 71, 79 and 80 have been canceled. To the extent that the rejection applies to the pending claims, Applicants respectfully traverse.

Under the Federal Circuit standard for enablement, some necessary experimentation by the skilled artisan is permitted; the amount of experimentation, however, must not be unduly extensive. *Atlas Powder Co. v. E. I. duPont de Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984). Furthermore, patent claims that include some claimed combinations

which are inoperative are not necessarily invalid under 35 U.S.C. § 112. *Id.* Factors to be considered when determining whether the amount of experimentation is undue were set out in *In re Wands*, 858 F.2d 731 at 737 (Fed. Cir. 1988).

Two factors to be considered in determining whether undue experimentation is required are "the amount of direction or guidance presented," and "the state of the prior art." *See Wands* 8 USPQ2d 1400 at 1404. In the present application, clear guidance on how to screen for agonists and antagonists of TNF family receptors is given, and the tools for carrying out the screens were readily available at the time of filing. At the time of filing, assays to screen for apoptosis, and for compounds which enhance or inhibit apoptosis, were well known in the art. Furthermore, a variety of these assays for determining DR4 activity (*e.g.*, cell death assays) are disclosed in the specification, *e.g.*, at page 14, line 35 through page 15, line 12; and at page 45, lines 13-38. The specification further discloses ways to adapt those cell death assays known at the time of filing or disclosed in the specification to screen for DR4 agonists or antagonists. *See, e.g.*, page 31, line 10 through page 32, line 12, and page 34, lines 2-9. These assays, and the techniques required are routine, and can be practiced easily by one of ordinary skill in the art. Furthermore, at the time of filing, the TNF-family ligand TRAIL had been isolated and characterized. *See* specification at page 3, line 22 through page 4, line 15, and the cited references therein. Accordingly, the TRAIL ligand was readily available for use in the screening assays. The Examiner has stated that "making a generic antibody is well within the skill of one in the art." (Paper Number 15, page 4.) Accordingly, given the materials available, the guidance provided in the specification, the knowledge in the art, and the high level of skill in the art, screening for agonist or antagonist activity of an antibody or fragment thereof would have been routine

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to one of ordinary skill in the art.

An additional factor to be considered in determining whether undue experimentation is required is "the quantity of experimentation necessary." *See Id.* With respect to screening populations of antibodies (*e.g.*, a plurality of monoclonal antibodies derived from a hybridoma library) for agonist or antagonist activity, the entire process would be considered by the skilled artisan as a single "experiment," much as the entire attempt to make a monoclonal antibody (*i.e.*, from a diverse population of hybridomas) is considered as a single "experiment " in *Wands*. *See* 8 USPQ2d 1400 at 1407. Furthermore, the skilled artisan would be prepared to screen antibodies which do not have agonist or antagonist activity, similar to the screening of large numbers of hybridomas discussed in *Wands*. *See Id.* at 1406. Thus, the possibility that some antibodies embodied by the claims would not function as an agonist or antagonist does not defeat enablement.

In view of these remarks, Applicants respectfully request that the Examiner reconsider and withdraw all rejections under 35 U.S.C. § 112, first paragraph, as applied to the pending claims.

Rejections under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 22, 24, 42, 44, 46, 64, 66, 73, 75, 82 and dependent claims 36-41, 58-63, 67-72, and 76-81 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. (Paper No. 15, page 5.)

Claims 22, 24, 42, 44, 46, 64, 66, 73, 75, 82 and dependent claims 36-41, 58-63, 67-72, and 76-81 have been canceled. As a way of making explicit that which was implicit in

the prior claims, new claims 100, 116, 134, 152, 174, 190, 208 and 232 recite that the immunogen comprises "an immunogenic epitope of the polypeptide consisting of amino acids 1-468 of SEQ ID NO: 2," or "an immunogenic epitope of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853." An "immunogenic epitope," as defined in the specification is "a part of a protein that elicits an antibody response when the whole protein is the immunogen." See page 27, lines 21-23 in the specification. Thus, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

The Examiner states that the use of the term "specifically binds" in claims 22, 24, 44, 46, 66 and 75 "is not clear since the specification does not define the term and it is not clear in this instance whether this limits antibodies to those that bind only DR4 of SEQ ID NO:2, any mammalian DR4, any DDR, etc." (Paper No. 15, page 5.) Claims 22, 24, 44, 46, 66 and 75 have been canceled. To the extent that the rejection applies to the pending claims, Applicants respectfully disagree.

Applicants submit that the use of the term "specific" with reference to antibody binding is one that is routinely used by persons of ordinary skill in the antibody arts. For example, the 1994 Boehringer Mannheim Biochemicals Catalog, uses the term "Specificity" to describe one of the many features of the antibodies which are offered for sale. For example, the catalog lists an Anti-Mac-1 (macrophage associated antigen) (clone M1/70) antibody and describes it under the heading "Specificity and Notes":

> The antibody *specifically* reacts with native mouse and human Mac-1 (complement receptor type 3; Ly-40) antigen and precipitates two chains, 170kD (CD11b) and 95kD (CD18).

(*See,* Boehringer Mannheim Biochemicals, Inc. 1994 Catalog, Page 260, attached hereto as

part of Exhibit A (emphasis added).) As a further example, an Anti-L-CAM/Uvomorulin (clone 6F9) antibody is described:

> The antibody *specifically* recognizes the 120 kD and the 80 kD band of L-CAM/Uvomorulin (Arc-1 E-cadherin cell-CAM 120/80) in man and rabbit. L-CAM/Uvomorulin staining is confined to the lateral border of epithelial cells and, within the intestine, shows more intense concentrations in the area of the junctional complex. As a positive control, the cell line MCF-7 can be used.

(*See,* Boehringer Mannheim Biochemicals, Inc. 1994 Catalog, Page 280, attached hereto as part of Exhibit A (emphasis added).)

Applicants submit that the term "specifically binds" or its equivalent "specifically recognizes" was clearly well accepted in the art at the priority date of the instant specification. The Catalog excerpts (*supra*) do not claim that the "specific" antibodies listed bind the desired protein to the exclusion of all other proteins, nor does it claim that they bind the desired protein to the exclusion of a similar protein in another mammal. However, as would be clear to one of ordinary skill in the art, these antibodies preferentially bind to their target antigen, or they would not be appropriate for their advertised use (*e.g.,* in the examples cited above, cryosections, Western (protein) blots, immunocytochemistry, or ELISA; and flow cytometry, immunocytochemistry, or immunoprecipitation, as shown on pages 260-261 and 280-281 of Exhibit A, respectively).

It is also well known to those of skill in the art that "[the] variable region, composed of 110-130 amino acids, give[s] the antibody its specificity for binding antigen" and that this binding occurs through the "hypervariable regions [that] directly contact a portion of the antigen's surface." (The Biology Project, Immunology, University of Arizona Online Tutorial www.biology.arizona.edu/immunology/tutorials/antibody/structure.html (2000)

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(visited February 14, 2002, attached hereto as Exhibit B). Therefore, the use of the term "specifically binds" in the present claims refers to an interaction of an antibody which preferentially binds, via its variable region, to a particular antigenic determinant present on a polypeptide of SEQ ID NO:2, or the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853.

Indeed, as far back as 1988, even the Court of Appeals for the Federal Circuit had a clear understanding of what "specifically binds" means when referring to antibodies. In *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988) the court used the term in referring to antibodies without further comment, because its meaning was so well understood.

> During a hepatitis B infection (or when purified HBsAg is injected experimentally), the body begins to make antibodies *that bind tightly and specifically* to HBsAg. Such antibodies can be used as regents for sensitive diagnostic tests (*e.g.*, to detect hepatitis B virus in blood and other tissues, a purpose of the claimed invention).

Id. at 733 (emphasis added). Thus, even among non-scientists, the term "specifically binds" as it applies to antibodies, was clearly understood, and its meaning was clear.

Given the well-known understanding of the term "specifically binds" as it applies to antibodies, Applicants submit that it would be routine for one of ordinary skill in the art to make and use an antibody or fragment thereof which specifically binds to DR4 or a polypeptide which shares, *e.g.*, 90% or more sequence identity with DR4.

Well known, routine assays for identifying a "specific" antibody were available at the priority date of the instant application. For example, *Current Protocols in Immunology*, a common laboratory handbook, provides, for example, three such assays: (1) Indirect ELISA to Detect *Specific* Antibodies; (2) Double Antibody-Sandwich ELISA to Detect

Specific Antibodies; and (3) Double-Immunodiffusion Assay for Detecting *Specific* Antibodies (*see, e.g., Current Protocols in Immunology* ed. Coligan *et al.* Vol. 2, Sections 2.1.1-2.1.20 and 2.3.1-2.3.3 (1991), attached hereto as Exhibit C (emphasis added). Clearly, the use of such assays was routine, and there was a clear understanding by those of ordinary skill in the art of antibodies as to the meaning of "specifically binds."

In view of these remarks, Applicants respectfully request that the Examiner reconsider and withdraw all rejections under 35 U.S.C. § 112, second paragraph, as applied to the pending claims.

Rejections under 35 U.S.C. § 103

The Examiner has rejected claims 22, 24, 36-42, 44, 46, 58-64, 66-73 and 75-82 under 35 U.S.C.§103(a) as allegedly being unpatentable over Chinnaiyan *et al.*, *Science* 274(5289):990-992 (1996) and Wiley *et al.* (U.S. Patent 5,763,223) in view of Lerner *et al.*, *Advances in Immunology* (36):1-45 (1984). Specifically, the Examiner has stated that

> Chinnaiyan et al. teach the DR3 polypeptide, a death domain-containing receptor (DDR), which has a sequence identical to the instant DR4 polypeptide of SEQ ID NO: 2 from amino acids 248-254, corresponding to amino acids 210-216 of DR3 the DcR3 polypeptide.

Id. Claims 22, 24, 36-42, 44, 46, 58-64, 66-73 and 75-82 have been canceled. As the rejection might apply to the pending claims, Applicants respectfully traverse. None of the references cited by the Examiner do not specifically teach an antibody which specifically binds to amino acids 219-216 of DR3 when taken alone, nor is there any suggestion to combine the references as the Examiner has done.

However, solely to expedite allowance of the claims, and not in acquiescence to the

Examiner's rejection, new claims 153 and 156 have been drawn to clarify that an isolated antibody or fragment thereof which specifically binds the full length polypeptide of SEQ ID NO:2 "binds to an antigenic epitope at least 9 amino acids long." Likewise, claims 209 and 212 have been drawn to clarify that an isolated antibody or fragment thereof which specifically binds the full length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853 "binds to an antigenic epitope at least 9 amino acids long." Support for "binding to an epitope at least 9 amino acids long" may be found, for example, at page 28, lines 5-8 in the specification. While not acquiescing to the Examiner's rejection, Applicants have drawn new claims 84, 101, 117 and 135 to an isolated antibody or fragment thereof which specifically binds the extracellular domain of the polypeptide of SEQ ID NO:2 (*i.e.*, amino acids 24 to 238) and new claims 159, 175, 191 and 215 to an isolated antibody or fragment thereof which specifically binds the extracellular domain encoded by the cDNA contained in ATCC Deposit No. 97853. Thus, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all currently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite

prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

The claims are amended as follows:

Claims 22-83 are cancelled.

Claims 84-248 are added.

The specification is amended as follows:

Beginning on page 5, line 24:

Figures [1A and 1B] 1A-1C show the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of DR4. It is predicted that amino acids 1-23 constitute the signal peptide, amino acids 24-238 constitute the extracellular domain, amino acids 239-264 constitute the transmembrane domain, and amino acids 265-468 constitute the intracellular domain of which amino acids 379-422 constitute the death domain.

Beginning on page 5, line 29:

Figures [2A and 2B] 2A-2C show the regions of similarity between the amino acid sequences of DR4, human tumor necrosis factor receptor 1 (SEQ ID NO:3), human Fas protein (SEQ ID NO:4), and the death domain containing receptor 3 (DR3) (SEQ ID NO:5).

Beginning on page 7, line 10:

Using the information provided herein, such as the nucleic acid sequence set out in Figures [1A and 1B] 1A-1C, a nucleic acid molecule of the present invention encoding a DR4 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention,

the gene of the present invention has also been identified in cDNA libraries of the following tissues: amniotic cells, heart, liver cancer, kidney, leukocyte, activated T-cell, K562 plus PMA, W138 cells, Th2 cells, human tonsils, and CD34 depleted buffy coat (cord blood).

Beginning on page 7, line 18:

The DR4 gene contains an open reading frame encoding a mature protein of about 445 amino acid residues whose initiation codon is at position 19-21 of the nucleotide sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO.1), with a leader sequence of about 23 amino acid residues (i.e., a total protein length of 468 amino acids), and a deduced molecular weight of about 50 kDa. Of known members of the TNF receptor family, the DR4 polypeptide of the invention shares the greatest degree of homology with human TNFRl and DR3 polypeptides shown in Fig. 2, including significant sequence homology over the multiple Cysteine Rich domains.

Beginning on page 8, line 14:

As indicated, the present invention also provides the mature form(s) of the DR4 protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit No. 97853, and as shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:2). By the mature DR4 protein having the amino acid sequence encoded by the cDNA clones contained in the host

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identified as ATCC Deposit No. 97853, is meant the mature form(s) of the DR4 protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature DR4 having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853, may or may not differ from the predicted "mature" DR4 protein shown in Figure 1 (amino acids from about 24 to about 468) depending on the accuracy of the predicted cleavage site based on computer analysis.

Beginning on page 9, line 32:

Isolated nucleic acid molecules of the present invention include DR4 DNA molecules comprising an open reading frame (ORF) shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1) and further include DNA molecules which comprise a sequence substantially different than all or part of the ORF whose initiation codon is at position 19-21 of the nucleotide sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1) but which, due to the degeneracy of the genetic code, still encode the DR4 polypeptide or a fragment thereof. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

Beginning on page 10, line 1:

In another aspect, the invention provides isolated nucleic acid molecules encoding the DR4 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97853 on January 21, 1997. Preferably, these nucleic acid molecules will encode the mature polypeptide encoded by the above-described deposited cDNA clone. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1) or the nucleotide sequence of the DR4 cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated DNA molecules and fragments thereof are useful as DNA probes

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for gene mapping by *in situ* hybridization of the DR4 gene in human tissue by Northern blot analysis.

Beginning on page 10, line 12:

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By fragments of an isolated DNA molecule having the nucleotide sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1) are intended DNA fragments at least 20 bp, and more preferably at least 30 bp in length which are useful as DNA probes as discussed above. of course larger DNA fragments 50-1500 bp in length are also useful as DNA probes according to the present invention as are DNA fragments corresponding to most, if not all, of the nucleotide sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1). By a fragment at least 20 bp in length, for example, is intended fragments which include 20 or more bases from the nucleotide sequence in Figures [1A and 1B] 1A-1C (SEQ ID NO:1).

Beginning on page 10, line 22:

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the DR4 extracellular domain (amino acid residues from about 24 to about 238 in Figure 1A (SEQ ID NO:2)); a polypeptide comprising the DR4 transmembrane domain (amino acid residues from about 239 to about 264 in Figure [1A] 1B (SEQ ID NO:2)); a polypeptide comprising the DR4 intracellular domain (amino acid residues from about 265 to about 468 in Figures [1A and 1B] 1B and 1C (SEQ ID NO:2)); and a polypeptide comprising the DR4 death domain (amino acid residues from about 379 to about 422 in Figure 1B (SEQ ID NO:2)). Since the location of these domains have been predicted by computer graphics, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 residues) depending on the criteria used to define the domain.

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Beginning on page 11, line 1:

Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding epitope-bearing portions of the DR4 protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 35 to about 92 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 114 to about 160 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 169 to about 240 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 267 to about 298 in Figure [1A] 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 330 to about 364 in [Figures 1A and] Figure 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 391 to about 404 in Figure 1B (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 418 to about 465 in [Figure] Figures 1B and 1C (SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the DR4 protein. Methods for determining other such epitope-bearing portions of the DR4 protein are described in detail below.

Beginning on page 11, line 16:

In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 as follows: HTOIY07R (SEQ ID NO:6) [,as shown in Figure 4A, and HTXEY80R (SEQ ID NO:7), as shown in Figure 4B] and HTXEY80R (SEQ ID NO:7) both shown in Figure 4.

Beginning on page 11, line 37:

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1) or Figures [2A and 2B] 2A-2C (SEQ ID NO:3).

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Beginning on page 12, line 3:

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3 terminal poly(A) tract of the DR4 cDNA shown in Figure [1B] 1C (SEQ ID NO:1)), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

Beginning on page 13, line 4:

Further embodiments of the invention include isolated nucleic acid molecules that are at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to (a) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figures [1A and 1B] 1A-1C (SEQ ID NO:2), including the predicted leader sequence; (b) nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figures [1A and 1B] 1A-1C (SEQ ID NO:2), including the predicted leader sequence but lacking the amino terminal methionine; (c) a nucleotide sequence encoding the mature DR4 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 24 to about 468 in Figures [1A and 1B] 1A-1C (SEQ ID NO:2); (d) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97853; (e) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader but lacking the amino terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853; (f) a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853; (g) a nucleotide sequence that encodes the DR4 extracellular domain, (h) a nucleotide sequence that encodes the DR4 transmembrane domain, (i) a nucleotide sequence that encodes the DR4 intracellular domain, (j) a nucleotide sequence that encodes the DR4 death domain; or (k) a nucleotide

sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above.

Beginning on page 14, line 4:

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figures [1A and 1B] 1A-1C or to the nucleotide sequences of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

Beginning on page 14, line 18:

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having DR4 activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having DR4 activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having DR4 activity include, *inter alia,* (1) isolating the DR4 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise

chromosomal location of the DR4 gene, as described in Verma *et al., Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting DR4 mRNA expression in specific tissues.

Beginning on page 14, line 32:

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs which do, in fact, encode a polypeptide having DR4 protein activity. By "a polypeptide having DR4 activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the DR4 protein of the invention (either the full-length protein or, preferably, the mature protein), as measured in a particular biological assay. For example, DR4 protein activity can be measured using the cell death assays performed essentially as previously described (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995); A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)) or as set forth in Example 5, below. In MCF7 cells, plasmids encoding full-length DR4 or a candidate death domain containing receptors are co-transfected with the pLantern reporter construct encoding green fluorescent protein. Nuclei of cells transfected with DR4 will exhibit apoptotic morphology as assessed by DAPI staining. Similar to TNFR-1 and Fas/APO-1 (M. Muzio, *et al., Cell 85*, 817-827 (1996); M. P. Boldin, *et al., Cell 85*, 803-815 (1996); M. Tewari, *et al., J Biol Chem 270*, 3255-60 (1995)), DR4-induced apoptosis is blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk.

Beginning on page 15, line 13:

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figures [1A and 1B] 1A-1C

(SEQ ID NO:1) will encode a polypeptide "having DR4 protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having DR4 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

Beginning on page 21, line 23:

The invention further provides an isolated DR4 polypeptide having the amino acid sequence shown in Figures [1A and 1B] 1A-1C [SEQ ID NO:2] or a peptide or polypeptide comprising a portion of the above polypeptides.

Beginning on page 26, line 3:

The polypeptides of the present invention also include the polypeptide encoded by the deposited cDNA including the leader, the mature polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figures [1A and 1B] 1A-1C (SEQ ID NO:2) including the leader, the polypeptide of Figures [1A and 1B] 1A-1C (SEQ ID NO:2) minus the amino terminal methionine, the polypeptide of Figures [1A and 1B] 1A-1C (SEQ ID NO:2) minus the leader, the extracellular domain, the transmembrane domain, the intracellular domain, the death domain, soluble polypeptides comprising all or part of the extracellular and intracelluar domains but lacking the transmembrane domain as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA clones, to the polypeptide of Figures [1A and 1B] 1A-1C (SEQ ID NO:2) and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

Beginning on page 26, line 31:

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figures [1A and 1B] 1A-1C (SEQ ID NO:2) or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

Beginning on page 27, line 4:

The present inventors have discovered that the DR4 polypeptide is a 468 residue protein exhibiting three main structural domains. First, the ligand binding domain was identified within residues from about 24 to about 238 in Figures 1A and 1B [SEQ ID NO:2]. Second, the transmembrane domain was identified within residues from about 239 to about 264 in [Figures 1A and] Figure 1B [SEQ ID NO:2]. Third, the intracellular domain was identified within residues from about 265 to about 468 in Figures [1A and] 1B and 1C [SEQ ID NO:2]. Importantly, the intracellular domain includes a death domain at residues from about 379 to about 422. Further preferred fragments of the polypeptide shown in Figures [1A and 1B] 1A-1C [SEQ ID NO:2] include the mature protein from residues about 24 to about 468 and soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain.

Beginning on page 28, line 9:

Non-limiting examples of antigenic polypeptides or peptides that can be used to

generate DR4-specific antibodies include: a polypeptide comprising amino acid residues from about 35 to about 92 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 114 to about 160 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 169 to about 240 in [Figure] Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 267 to about 298 in Figure [1A] 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 330 to about 364 in [Figures 1A and] Figure 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 391 to about 404 in [Figure] Figures 1B and 1C (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 418 to about 465 in Figure [1B] 1C (SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the DR4 protein.

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